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ESSAY

Differences between non-specific and bio-specific, and between equilibrium and non-equilibrium, interactions in biological systems

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Abstract. The interaction forces between biological molecules and surfaces are much more complex than those between non-biological molecules or surfaces, such as colloidal particle surfaces. This complexity is due to a number of factors: (i) the simultaneous involvement of many different molecules and different non-covalent forces – van der Waals, electrostatic, solvation (hydration, hydrophobic), steric, entropic and 'specific', and (ii) the flexibility of biological macromolecules and fluidity of membranes. Biological interactions are better thought of as 'processes' that evolve in space and time and, under physiological conditions, involve a continuous input of energy. Such systems are, therefore, not at thermodynamic equilibrium, or even tending towards equilibrium. Recent surface forces apparatus (SFA) and atomic force microscopy (AFM) measurements on supported model membrane systems (protein-containing lipid bilayers) illustrate these effects. It is suggested that the major theoretical challenge is to establish manageable theories or models that can describe the spatial and time evolution of systems consisting of different molecules subject to certain starting conditions or energy inputs.

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Biological interactions are 'processes'

Most chemical and colloidal interactions are considered as occurring within a single macromolecule (e.g. a folding protein), between a molecule and its surrounding medium (solvent effects), or among pairs of molecules, particles and surfaces (binding, aggregation and adsorption). However, under physiological conditions, biological interactions are 'many-body' interactions that progress in space and time. A good example is the saltatory motion of white blood cells

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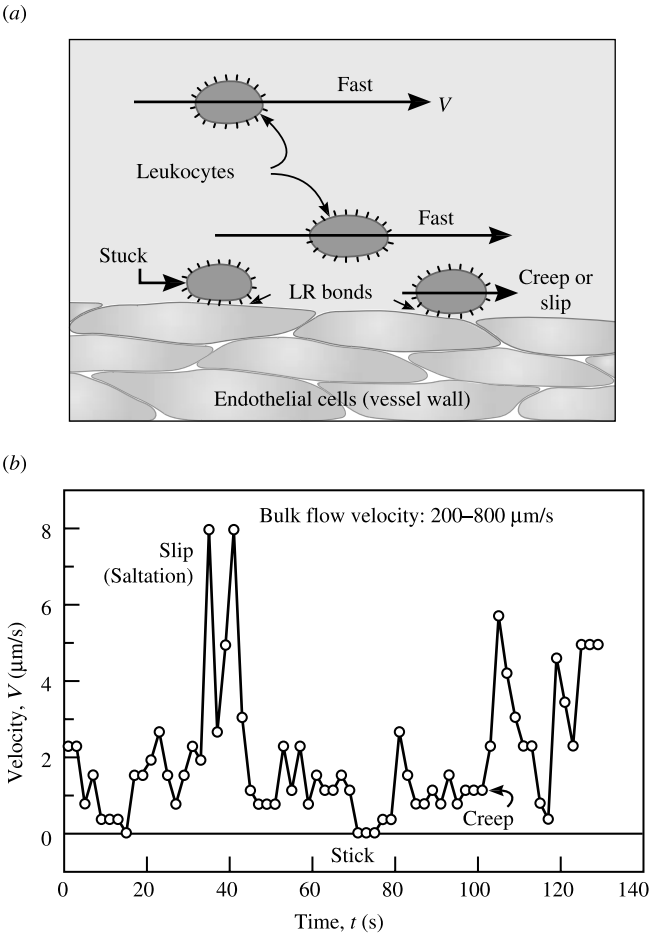


Fig. 1. 'Leukocyte rolling' along the endothelium *in vitro* (*a*) involves sticking ($V=0$), 'rolling' or 'creep' ($V\approx 1$ μm/s), slip or saltation ($V\approx 5$ μm/s), and free or bulk flow ($V=200$–800 μm/s). Each rolling process involves many mobile molecules on both surfaces that interact via LR bonds that in turn form and break in a complex but well-orchestrated way.

along the endothelium surfaces of blood capillaries (Fig. 1*a*), which follows a chaotic, but not random, stick–slip type motion (Fig. 1*b*).

This behaviour has been successfully modelled in terms of the thermally controlled binding and unbinding of specific ligand-receptor (LR) bonds on the two surfaces by Dan Hammer and co-workers. Interestingly, the theoretical model used is conceptually similar to models used to analyse stick–slip friction, sound generation (e.g. the sound of a squeaking door or a violin), seismic movements and earthquakes.

Intermolecular forces involved

Figure 2 shows some of the more common intermolecular and interparticle pair potentials, although the interactions or processes occurring between biomolecular assemblies such as those

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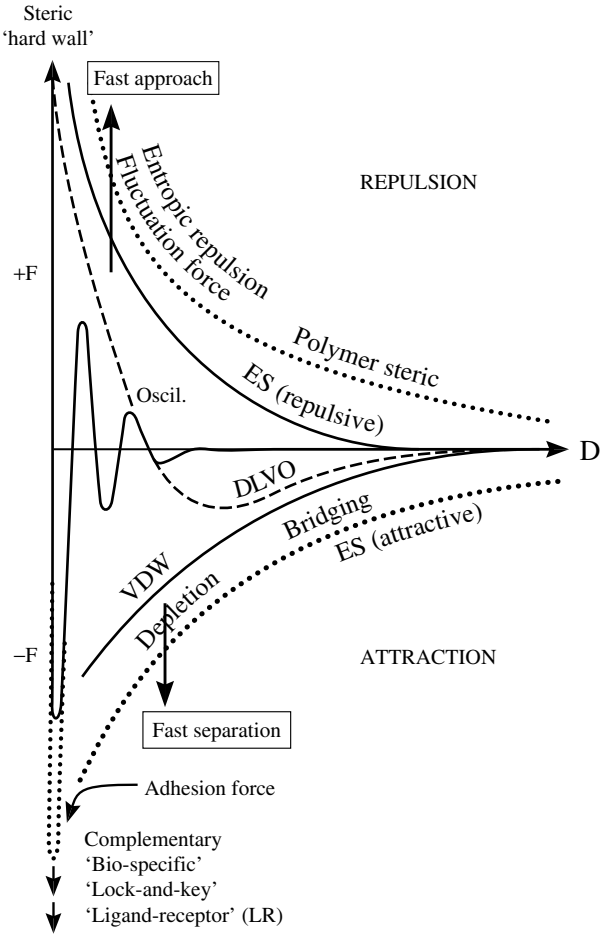


Fig. 2. Different types of pair potentials or force-laws between macromolecules, small particles and surfaces. Biological molecules and membrane surfaces often interact via four or more of these attractive and repulsive forces either simultaneously or following in some systematic order. (Oscillatory forces due to solvent structure occur between smooth hard surfaces and are rarely, if ever, encountered in biological systems.)

shown in Fig. 1 are far more complicated than can be described by these potentials because, as will be illustrated below, they are not additive in space and usually act at different times.

Synergy between different forces occurring at different locations

Biological cells have clearly developed means for controlling different interaction forces over different distance and timescales. For example, a transient change in the local pH or calcium ion concentration at a membrane surface will modify the *long-range* electrostatic interaction but may not affect the *short-range* LR interaction. A change in the receptor-binding-site affinity could alter the short-range force but not the long-range double-layer force. Moreover, a change in the local membrane fluidity will alter the lateral diffusion rate of a ligand, thereby preventing or enhancing its probability of capture by a receptor.

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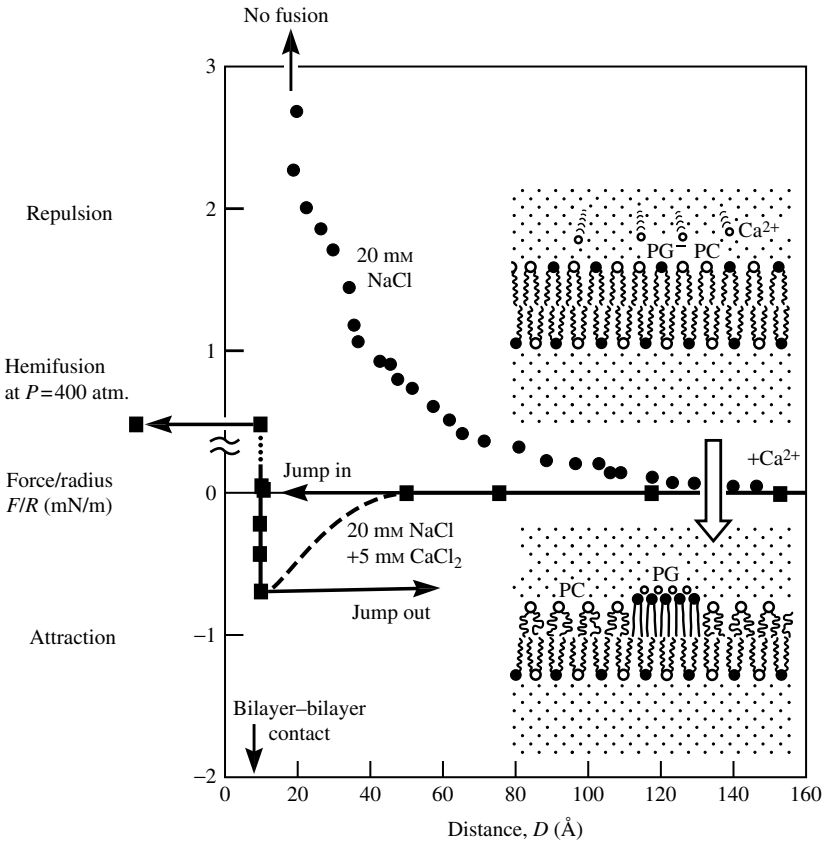


Fig. 3. Example of the space–time evolution of a simple fusion event or process. Measured forces (by Leckband and co-workers) between mixed anionic/uncharged $DLPG^-$/DLPC bilayers in 20 mM NaCl with and without added 5 mM $CaCl_2$. The force in NaCl solution (without calcium) is dominated by a double-layer repulsion and is similar to the force between other pure (one-component) anionic bilayers. No adhesion is observed nor can fusion be induced between such bilayers. In contrast, the force in the presence of calcium is completely different from the forces between either pure DLPC or pure $DLPG^-$ bilayers. With the mixed lipid bilayers, calcium-induced phase separation (clustering) of the negatively charged PG^- lipids separates the charged from the uncharged lipids, as shown in the lower inset. The stressed DLPC regions become more hydrophobic and act as adhesion and fusion sites with other, similarly stressed, bilayers.

The opening up or exposure of hydrophobic groups can be triggered by a number of stress-inducing effects such as a change in the conformation of a protein, a local change in the curvature of membrane lipids, calcium-ion binding to one side of a membrane, trans-membrane electric fields, flow stresses, and changes in the trans-membrane osmotic pressure. There is now ample evidence that the exposure of hydrophobic 'pockets' leads to adhesion and fusion, both of proteins and lipid bilayers. Moreover, the stresses that induce these pockets can occur well away from the pockets and, therefore, from the adhesion or fusion site. For example, an osmotic pressure change triggered in a vesicle by a particular protein will cause water to flow into the vesicle interior. This will cause the vesicle to swell, increasing the tension of the entire membrane that might result in fusion (e.g. with another membrane) in a completely different place on the vesicle surface.

An example of how hydrophobic and electrostatic interactions between mixed lipid bilayers can work together to induce bilayer–bilayer fusion is illustrated in Fig. 3. The top half shows the

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bilayer structure and measured inter-bilayer forces in monovalent electrolyte; the lower half shows the effects of adding calcium ions to the solution which caused adhesion and easy hemi-fusion (a schematic of two hemi-fused bilayers is not shown). The effects of calcium on the mixed lipid bilayers are quite different from those induced on the pure, single-component DLPC or $DLPG^-$ bilayers. With mixed bilayers, lateral phase separation or clustering occurs where PG^- lipids are pulled close together (condensed) by calcium binding. The condensed PG–calcium domains result in a tensile stress on the remaining PC-rich regions, which become more hydrophobic and thereby act as adhesion and/or fusion sites. In this system, fusion would not occur unless a number of favorable factors are present simultaneously: mixed rather than single-component bilayers, divalent counterions on one side of the bathing solution only, and fluid membranes that allow molecular rearrangements to occur. Note, too, that with this type of fusion mechanism, fusion will occur at a different location from the calcium-binding sites. In addition, in a living system, unlike the model system shown in Fig. 3, the calcium ions that trigger the fusion event are likely to be released as a transient spurt, so that after some (short) time they will redisperse in the bathing solution, bringing the system back into its quiescent state, i.e. one or a few fusion events will occur, rather than a continuous build-up of fusion sites.

The above example applies to lipid bilayers but applies, in principle, to other biological systems. The role of calcium, and to a lesser extent magnesium, in triggering membrane fusion is generally thought to occur more commonly via their effect on protein structure, which causes them to expose hydrophobic domains, rather than by inducing structural changes in the lipids. The membrane fusion channels opened up by soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins at vesicle–presynaptic junctions when calcium is released into the junction may be an example of this type of mechanism involving both lipids and proteins.

Non-equilibrium, rate and time-dependent interactions

In a classic paper in *Science* in 1972 George Bell quantitatively analyzed the role of thermal fluctuations on the measured force needed to unbind or separate two adhering molecules (or a lipid or protein from a membrane surface). He showed that even as the interaction potential is a well-defined function of space, the separation force is not, but depends on the length or size of the 'bond', the temperature, the pulling speed and waiting time: the slower the pulling speed or the longer the waiting time the lower the separation or 'adhesion' force. Such forces have been measured using both the atomic force microscope and surface forces apparatus techniques, mainly between ligands and their receptors, but also in the unfolding of proteins and nucleic acids. The important conceptual message here is that separation or unbinding is determined by a statistically rare event.

Since separation is determined by a number of factors that are likely to be different for different types of binding interactions, when a number of bonds occur in series, they can break in one place at one pulling speed, but break at another place at a different pulling speed. The quantitative measurement and analysis of multiple bonds in biological systems, including bonds in series and bonds in parallel, remains an important but unsolved problem for both experimentalists and theoreticians.

Equally interesting and subtle, is the reverse process of 'binding', 'capture' or 'recognition', which again is often determined by a statistically rare event. This is illustrated in Fig. 4 for the case of a surface consisting of tethered ligands approaching or moving past a surface covered with receptors (cf. Fig. 1).

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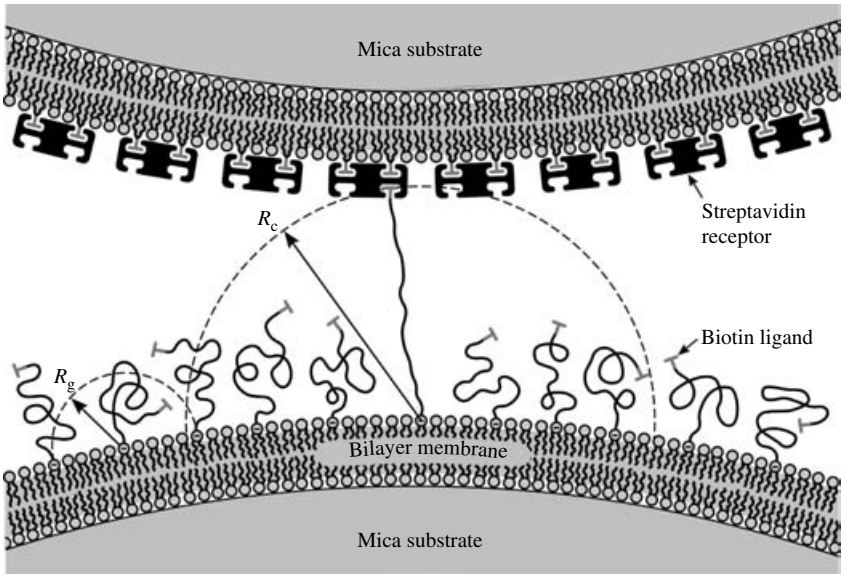


Fig. 4. The probability of a successful binding of a tethered ligand to a receptor at a large distance away (the capture distance, R_c) increases with the waiting time and depends more on the dynamics of the tether (polymer dynamics) than the binding energy. As in the case of separation, capture depends on a statistically rare event. The 'statistics' of the whole process involves a knowledge of single-molecule dynamics, the spatial distribution and lateral diffusion of the molecules (many-molecule dynamics), the shapes and relative motion of the two surfaces (macroscopic structure and dynamics), the LR binding energies, and other factors. [Figure adapted from Wong and co-workers, 1997, and Jeppesen and co-workers, 2001.]

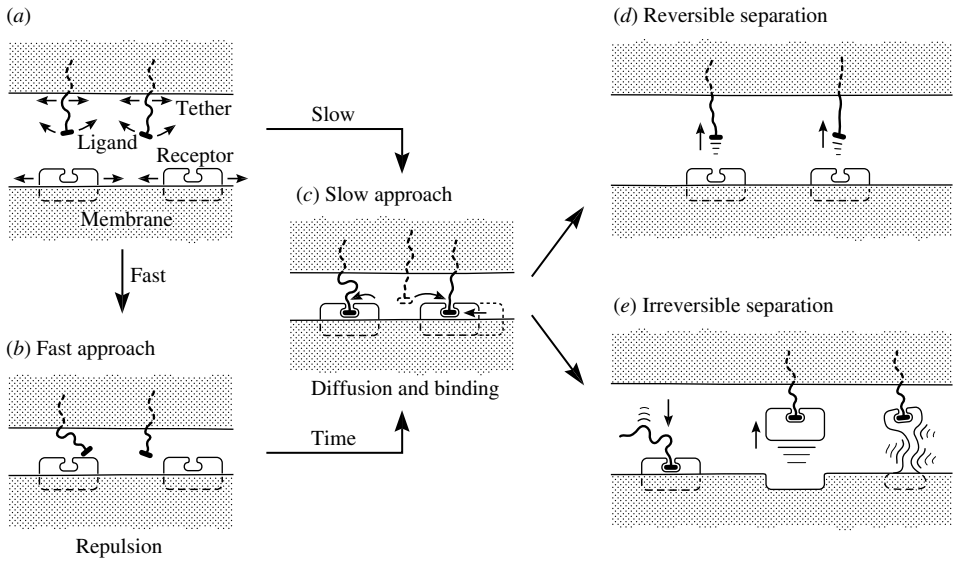


Fig. 5. On fast approach (*a*)→(*b*) there is an additional kinetic force barrier because the ligand does not have time to find the binding site until some finite time after the surfaces have been close together, (*b*)→(*c*). On separation, depending on the relative bond energies, bond lengths, and the rate of separation, the original LR bonds may break (reversible separation), or lipid molecules may be pulled out of the membrane, or the receptor molecules may be pulled out, or the protein may unfold (irreversible separation). Other scenarios are also possible, including statistical combinations of the above.

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Reversible and irreversible interactions

When considering a repetitive or cyclic process, such as the approach *and* separation of two molecules, or the continuous flow of cells past each other (cf. Fig. 1*a*), subtle and unintuitive things can happen. Examples of some possible scenarios are illustrated in Fig. 5, where two membranes – one exposing tethered ligands, the other with receptor proteins – come together then separate. Such processes are rarely reversible, either energetically or structurally, and many quite different scenarios can arise depending on the relative values of the bond energies and bond lengths of the LR bond, the lipid–membrane 'bond', the protein–membrane 'bond' and the internal bonds holding the protein receptor together. In addition, the tether length, the temperature, the fluidity of the membrane lipids, and particularly the approach and separation rates, all play important roles. Thus, in Fig. 5, we find that on fast approach there may be no binding (*a*)→(*b*), whereas on slow approach there is (*a*)→(*c*) or (*b*)→(*c*). On separation, again depending on the factors mentioned above, the bonds could break at their original sites (*d*), but they could also break elsewhere including the unraveling of the protein as often occurs in AFM pulling experiments. One interesting possibility, which no doubt occurs in some biological interactions, is the transfer of a molecule, via binding and pull-out, from one vesicle or cell membrane to another as the two pass each other, as exemplified by the first two diagrams of Fig. 5*e*.

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